continuously adjust its plasticity threshold according to its recent global activity level, keeping the overall level of synaptic drive within a range that allows expression of plasticity. In this sense, the metaplasticity ought to be a transient, cell-wise mechanism. For how long does the neuron need to integrate its firing activity in order to determine its future plasticity threshold? How long does metaplasticity induced by a bout of prior activity persist? How does activation of NMDA receptors lead to a change in plasticity threshold? By altering the function or trafficking of NMDA receptors themselves, or the functional state of kinases and phosphatases central to plasticity processes (Abraham, 2008)? Or membrane voltage-dependent conductances are altered so that the voltage threshold for plasticity is affected? Future experiments will help elucidate the mechanisms underlying metaplasticity.

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A Stretch from the Periphery Helps Brain Clocks Feel the Daily Heat

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In this issue of *Neuron*, Sehadova et al. show that synchronization of circadian clocks in the brains of *Drosophila* by daily temperature changes requires chordotonal organs, mechanosensory structures that function as stretch receptors in insects. This is strikingly different from the more direct path by which brain clocks perceive light.

Circadian (≅24 hr) pacemaker neurons situated in the brain are usually considered the central clocks in animals as they drive what is undoubtedly the behavioral rhythm at the top of the hierarchy in the temporal organization of an animal's life, namely its daily wake-sleep cycle. Light-dark cycles resulting from the Earth's rotation on its axis are likely the main environmental cue in nature that synchronizes (entrains) most, if not all, circadian clocks to local time. However, diurnal changes in temperature are also potent synchronizers of circadian rhythms in many life forms, from bacteria to plants and animals (Rensing and Ruoff, 2002). Strong progress has been made toward understanding the cellular and molecular bases for circadian photoentrainment in diverse model organisms, but how clocks are synchronized by daily temperature cycles is much less clear. Using *Drosophila melanogaster* as a model system, Sehadova et al. (2009) report in this issue of *Neuron* that the way a brain clock "sees the light" and "feels the heat" can exhibit some remarkable differences. While light is thought to directly penetrate through the head into the fly brain, where it stimulates photoreceptors expressed in clock cells, the authors show that chordotonal organs in the periphery, specialized structures involved in insect sensory mechanotransduction (Kernan, 2007), are required to transduce daily temperature cues to brain clocks. Thus, mechanoreceptor neurons in insects not only function in such fundamental senses as hearing and proprioception (Kernan, 2007) but might also regulate the *timing* of behavioral programs by working as thermal sensors.

A role for temperature in modulating circadian systems would seem especially relevant in poikilothermic organisms like *Drosophila*. Indeed, flies exposed to daily temperature cycles (e.g., 12 hr at 16°C followed by 12 hr at 25°C) manifest one main activity component during the warm

phase that peaks prior to (i.e., "anticipates") the beginning of the cryophase. This is similar to, but distinct from, the more bimodal distribution observed in standard light-dark cycles (such as 12 hr of light followed by 12 hr of dark), with bouts centered on sunrise ("morning" peak) and sunset ("evening" peak).

Findings described in this issue build on prior successes using transgenic *Drosophila* whereby the *luciferase* (*luc*) open reading frame is placed downstream of promoter elements that drive daily cycles in the expression of central clock genes, such as *period* (*per*). With this experimental approach it is possible to assay intracellular oscillator function in live animals in a noninvasive manner by measuring bioluminescent rhythms emanating from the *luc*-based reporters (Plautz et al., 1997). When first applied, this strategy led to the surprising realization that autonomous circadian clocks capable of being entrained by light-dark cycles are located throughout the body of the fly and not just the brain (Plautz et al., 1997). Further insights into the mechanisms underlying photic entrainment were gained by the identification of CRYPTOCHROME (CRY), the major circadian-relevant photoreceptor in flies (Stanewsky et al., 1998). Although it is not fully understood how CRY works in circadian photoreception, light stimulation of CRY leads to the rapid degradation of TIMELESS (TIM), a central player in the circadian clock mechanism (Ashmore and Sehgal, 2003). The light-induced degradation of TIM acts as a reset that synchronizes clocks to the prevailing daily light-dark cycle. Thus, throughout the fly body, clock cells that express *cry* (not all of them do, see below) are directly photosensitive (see Figure 1).

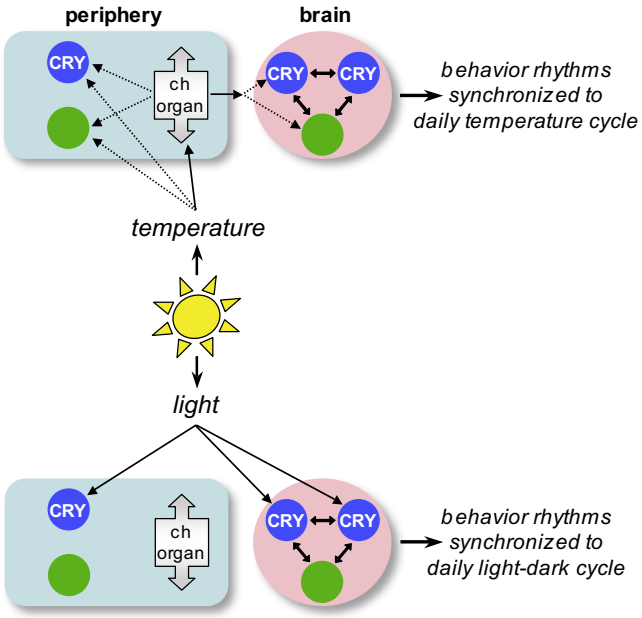

Figure 1. Distinct Pathways Underlie How Diurnal Changes in Temperature and Light Synchronize Behavioral Rhythms in *Drosophila*

For simplicity, two types of clock cells are shown, those that express CRY (blue circles) and are directly photosensitive and those that do not express CRY (green circles). In the brain, clock neurons participate in an integrated network that drives rhythmic behavior. Synchronization of brain clocks by daily changes in temperature requires the ch organs, which normally function as sensory stretch receptors. It is not clear if all brain clocks are equally responsive to the thermal signals transduced by the ch organs and if ch organs are required for the temperature entrainment of peripheral clocks (broken arrows).

Several years ago, Glaser and Stanewsky (2005) applied the bioluminescent imaging strategy to begin dissecting the temperature entrainment pathway in *Drosophila*. What they found seemed very similar to earlier work on circadian photoreception, whereby a wide variety of explanted tissues exhibit oscillator function that can be synchronized by temperature cycles, suggesting that thermal entrainment is a largely tissue-autonomous property. They also reported the isolation of a mutation termed *nocte* (*no circadian temperature entrainment*) that appears to specifically abolish/attenuate synchronization of molecular and behavioral rhythms to temperature cycles but not light-dark cycles.

Now, Sehadova et al. (2009) make a slight adjustment from their earlier work that has big implications. Upon closer inspection it was realized that brain clocks in isolated heads do not exhibit bona fide entrainment to temperature cycles. The bioluminescent rhythms observed in these preparations are merely driven by the imposed temperature fluctuations, peaking just after the start of the warm phase, essentially antiphase to normal *per* RNA cycles. Curiously, this rhythm that even persisted in brains isolated from clock mutant flies were not even coming from known clock cells but ectopic locations. Why can brain clocks exhibit oscillations in clock gene expression that are synchronized to daily temperature cycles when assayed in intact flies but not when evaluated in isolation? Is there something peripheral to heads that is required?

As a means to address these issues, they cloned *nocte*. Nocte is predicted to encode a rather large protein of over 2300 aa and is found in insects but without obvious vertebrate homologs. Unfortunately, not much molecular insight was gained from sequence analysis. However, its site-of-action with regards to circadian temperature entrainment led to unanticipated insights. Evidence based on using the UAS/GAL4 system to drive tissue-specific target gene expression suggested that peripheral mechanosensory organs are involved in the temperature entrainment of brain-driven behavioral rhythms. Attenuating *nocte* expression by RNA interference (RNAi) in cells where the *F-gal* driver is active, which includes the chordotonal (ch) and external sense (es) organs (such as sensory bristles) among other tissues, led to severe deficits in the temperature entrainment. Moreover, flies with mutations in genes known to affect the function of ch organs but retain normal bristle function, such as *tilB* (*touch insensitive larvaeB*), identified the ch organs as the major or sole sensory mechanotransduction structures in the temperature entrainment of behavioral rhythms.

How might ch organs sense daily changes in temperature? The authors propose a model based on recent findings that explore the relationship between the structure and function of ch organs. Chordotonal organs are attached to the

underside of the cuticle where they are found at nearly every joint within limb and body segments of insects (Kernan, 2007). A major structural unit of ch organs is the scolopale, a fluid-filled capsule that encloses the ciliary sensory endings of 1–3 neurons and is attached at their tips to an extracellular dendritic cap. Mechanical stimulation that leads to stretching of the sense organ stimulates the neurons, converting the physical signal into neuronal receptor potentials. To date, these mechanotransduction receptors are implicated in sensing several physical stimuli, such as sound, gravity, and wind that impact many complex behaviors in the adult fly (Eatock, 2009; Kernan, 2007). Relevant to the findings of Sehadova et al. (2009), *Drosophila* mechanoreceptors are very resistant to cellular deformation caused by elevated temperatures (e.g., 37°C), enabling them to maintain diverse sensory integrity despite random fluctuations in temperature normally encountered in natural settings (Cook et al., 2008). Interestingly, mutations in the Spam protein weaken resistance to cellular deformation of mechanosensory organs at elevated temperatures, severely impairing their function (Cook et al., 2008). Presumably, in the absence of protective mechanisms elevated temperatures trigger an osmotic imbalance that leads to excessive water loss from the liquid-filled scolopale capsules causing a collapse in the structural integrity needed to function.

Sehadova et al. (2009) show that *spam* mutants also have defective temperature entrainment of behavioral rhythms. In the case of *nocte*, it is highly expressed in ch organs (at least as assayed by a reporter construct), but also has a wide spatial distribution, including the brain. Although the role of *nocte* in the brain is not known, its presence in ch organs suggested it has a rather direct role in the function/structure of these sensory units. Indeed, mutations in *nocte* appear to diminish the structural (or functional) integrity of the dentritic cap, possibly opening a route for excessive water loss and hence cellular deformation at higher temperatures. Moreover, just like *spam* and other mutants that affect mechanotransduction, *nocte* mutants show a moderate uncoordinated phenotype at elevated temperatures.

Although the findings provide a plausible model for how mutations in *nocte* can impair ch organ function, it remains unclear if less extreme and lower amplitude temperature cycles encountered in natural conditions can evoke calibrated changes in mechanosensory neuron activity by regulating cell shape via temperature-induced changes in water content. A possible problem with this model is that for the system to convey time-of-day information it must be able to interpret *relative* changes in temperature and not merely function as a thermometer. For example, how does the fly "know" that 25°C is the warm part of the day in a 20°C/25°C cycle but the cold part of the day in 25°C/29°C cycle? Might thermoTRPs, cation channels that respond to different temperature ranges, play a role in identifying cold and warm phases (Patapoutian et al., 2003)? More work will be required to understand the basis for the thermal integration.

In addition, there are several other key issues that are not yet resolved (see Figure 1). Among them is how the daily thermal signals are transduced from ch organs to the brain clocks. *Nocte* expression is not rhythmic, and genetically abolishing the function of peripheral clocks does not impair the entrainment of behavioral rhythms to daily temperature cycles. Are some brain pacemaker neurons primary targets of the incoming temperature signals? Increasing evidence indicates that the ~150 individual pacemaker neurons in the adult brain fall into rhythmic clusters with distinct functional attributes that interact to generate a complex circadian neural circuitry (Nitabach and Taghert, 2008). In related work, it was shown that although temperature entrainment of behavioral rhythms also requires the circadian pacemaker network in the brain, there are some clock cells that seem more sensitive to temperature changes compared to light-dark cycles, perhaps because they do not express CRY (Dubruille and Emery, 2008). Are the daily temperature signals sensed by the ch organs first transduced to thermally sensitive brain clocks that subsequently synchronize other pacemaker neurons via network properties of the circadian circuit? For that matter, are ch organs required to synchronize peripheral clocks? And finally, are there specific ch organs that are the biologically relevant thermal transducers to the circadian system?

In thinking about the findings of Sehadova et al. (2009), one is left with the "why" question that is always so difficult to address. Why go through such an indirect route to synchronize brain clocks to diurnal changes in temperature when photic cues apparently bypass all this and just waltz in to light up the inside of the brain? The authors speculate that a brain pacemaker circuit designed with little intrinsic responsiveness to temperature changes yet sensitive to signals from an offsite thermal integrator would minimize undesired perturbations in clock dynamics by random fluctuations in temperature (such as intermittent clouds) while maintaining the ability to be synchronized by the more protracted diurnal temperature cycles. There is some precedence for this, as it seems that inactivating *cry* or "weakening" the brain circadian network makes behavioral rhythms more sensitive to daily temperature changes (Dubruille and Emery, 2008). Intriguingly, in mammals the central circadian pacemaker in the brain also appears less influenced by temperature cycles compared to clocks in peripheral tissues (Brown et al., 2002). However, this also begs the question as to the physiological influence of daily temperature cycles on circadian systems in natural settings where light-dark cycles are present. It is also important to note that not all the effects of temperature on circadian clocks are mediated by the same mechanism. For example, the daily distribution of activity in *Drosophila* adapts to seasonal changes in temperature by a mechanism involving the thermal sensitive splicing of a 3′-terminal intron in *per* (Majercak et al., 1999). Prior work showed that this splicing event has little to no role in the synchronization of behavioral rhythms to diurnal changes in temperature (Glaser and Stanewsky, 2005).

Thus, the role of temperature on circadian systems is almost certainly more complex compared to light. There is much yet to learn, but the findings by Sehadova et al. (2009) provide surprising insights into the diversity of circadian entrainment pathways and reveal an unexpected role for mechanosensory organs in regulating the *timing* of complex behaviors.

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